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TITLE: Application of FDA-Approved Memantine and Newer NitroMemantine Derivatives to Treat Neurological Manifestations in Rodent Models of Tuberous Sclerosis Complex

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The quality of life for those afflicted by Tuberous Sclerosis Complex (TSC) is affected by intellectual and neurological disabilities mediated in part by excessive glutamatergic activity in the brain. It is important to develop rational and effective therapies for early postnatal or even embryonic treatment of these neurological manifestations. Towards this goal, we propose to investigate if administration of the FDA-approved drug, Memantine, an uncompetitive/fast off-rate antagonist of the Nmethyl-D-aspartate-type glutamate receptor, and its improved derivative, NitroMemantine, ameliorate neurological complications in mouse models of TSC. During Year 01 of the grant, we obtained favorable results for Memantine on electrophysiological and neurobehavioral tests in Tsc2+/- mice. Treatment of Tsc2+/- mice with Memantine improved long-term potentiation (LTP), an electrical correlate of learning and memory, in the CA1 region of the hippocampus. Additionally, treatment of Tsc2+/- mice with Memantine showed a trend towards restoration of the ability to locate the platform in the Morris water maze, a neurobehavioral test of hippocampal memory. In Year 02, we further validated and extended our findings with Memantine and critically tested the efficacy of NitroMemantine. We expected that NitroMemantine will offer an advantage over Memantine in TSC model mice, as we have previously demonstrated in other neurological disorders.

15. SUBJECT TERMS

Excessive glutamatergic activity, Extrasynaptic NMDA receptors, Uncompetitive antagonist of NMDA receptors, Memantine, NitroMemantine

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FINAL SCIENTIFIC REPORT FOR W81XWH-13-0053

1. INTRODUCTION:

Tuberous sclerosis complex (TSC) is caused by heterozygous mutations that inactivate one of two genes, *TSC1* or *TSC2*. The central nervous system is affected in TSC, and neurological manifestations include intellectual disabilities, neurobehavioral abnormalities such as autism, and epilepsy. Currently, there is no cure for TSC. The mechanism of pathogenesis is not entirely clear, but TSC-related neurological symptoms are accompanied by excessive glutamatergic activity and altered synaptic spine structures. Our group previously developed, characterized, and licensed patents for the drug Memantine (Namenda®), which is now FDA approved for treatment of moderate-to-severe Alzheimer's disease, showing that it is an uncompetitive/fast off-rate antagonist of the *N*-methyl-D-aspartate-type glutamate receptor (NMDAR). Interestingly, Memantine is also in clinical trials for children with epilepsy, intellectual disabilities, and autism. Additionally, we have recently developed a new series of derivatives, termed NitroMemantines, with superior efficacy to Memantine both *in vitro* and *in vivo*. Our recent work has demonstrated that Memantine and NitroMemantine selectively block excessive activity of extrasynaptic NMDARs (eNMDARs) and protect synaptic integrity. Furthermore, our phosphoproteomic analysis of eNMDAR-mediated signaling has identified upregulation of the p38 MAPK (mitogen-activated protein kinase)-MAPKAPK2 (mitogen-activated protein kinase-activated protein kinase 2) cascade. Since this pathway is known to inactivate TSC2, inhibition of eNMDARs with Memantine or NitroMemantine should increase TSC2 activity. Therefore, we hypothesized that this action of Memantine and NitroMemantine may offer a rational treatment for individuals with TSC. To begin to test this hypothesis, we thus proposed the following specific aim:

1. To investigate if Memantine or NitroMemantine administration improves synaptic spine structure, electrophysiological properties, and behavioral abnormalities in TSC murine models by affecting the eNMDAR/p38 MAPK/ MAPKAPK2/TSC2 cascade.

We proposed to examine if excessive eNMDAR activity and its downstream signaling cascade, which inhibits TSC2 activity, is a pathogenic mechanism in the development of synaptic spine abnormalities, electrophysiological abnormalities, and neurobehavioral manifestations in TSC. Also, we are investigating if the FDA-approved drug Memantine or the new improved derivative that we have synthesized, NitroMemantine, can alleviate synaptic spine and behavioral abnormalities in TSC murine models. Thus, this proposal will yield further mechanistic insight into the disease and evaluate new potential pharmaceutical approaches to treatment.

2. KEYWORDS:

Epilepsy, Intellectual disability, Autism, Excessive glutamatergic activity, Extrasynaptic NMDA receptors, Memantine, Uncompetitive antagonist of NMDA receptors, p38MAPK, MAPKAPK2, mTOR, p70 S6 kinase

3. OVERALL PROJECT SUMMARY:

Task 1. We tested the effect of Memantine vs. NitroMemantine in the *Tsc1*$^{+/-}$ and *Tsc2*$^{+/-}$ mouse models of TSC, including electrophysiological, histological, biochemical, and behavioral readouts (months 1-24)

Results

To fulfill the aim of this grant, we had proposed to obtain *Tsc2* knockout mice from Jackson Laboratory. However, since the Jackson Laboratory possessed only frozen embryos, it was expected to take several months to have *Tsc2* knockout mice from that source. Thus, we instead obtained *Tsc2*$^{+/+}$ and

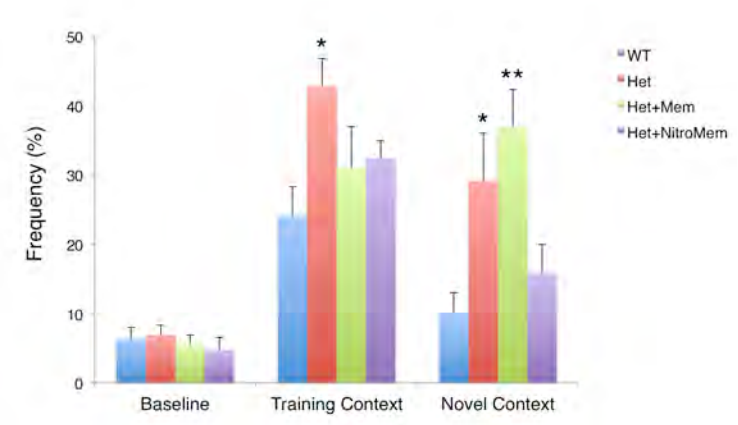

Fig. 1. Memantine and NitroMemantine reversed fear conditioning deficits in *Tsc2*$^{+/-}$ mice
Tsc2$^{+/+}$ mice (WT) or *Tsc2*$^{+/-}$ mice were treated with, vehicle, Memantine (Mem), or NitroMemantine (NitroMem) for two days and analyzed for fear conditioning tests. **P* < 0.05, ***P* < 0.01 (ANOVA with Dunnett's test, *n* = 6-7 mice per group)

Tsc2$^{+/-}$ mice from Dr. Mark F. Bear (MIT) (1). These mice are on the C57Bl/6J clonal background (1).

We first performed a behavioral readout, the fear conditioning test, on three month-old *Tsc2*$^{+/+}$ and *Tsc2*$^{+/-}$ mice to explore the effects of Memantine and NitroMemantine on anxiety, learning, and memory deficits in *Tsc2*$^{+/-}$ mice (2), as we had proposed. We administered Memantine or NitroMemantine intraperitoneally twice daily for 2 days, and 2 hours before the training session. As shown in **Fig. 1** (on previous page), *Tsc2*$^{+/-}$ mice showed excessive freezing responses in training and novel contexts. Remarkably, Memantine treatment mitigates the responses in the training context and NitroMemantine reversed the extreme responses in both training and novel contexts.

Next, we examined LTP (long-term potentiation) using field recordings in the CA1 region of hippocampal slices to investigate the effects of these mutations on synaptic plasticity. We prepared acute hippocampal slices from one-month old mice. We performed field recordings in an MEA (microelectrode array) chamber perfused with ACSF (artificial cerebrospinal fluid). We recorded fEPSPs (field excitatory postsynaptic potentials) in the hippocampal CA1 region with MEA. We evoked LTP by stimulation of the Schaffer collaterals (four repetitions of 100 Hz pulses for one sec each). The initial slope of the fEPSP was analyzed to assess synaptic plasticity. As shown in **Fig. 2A**, we confirmed abnormality of LTP in *Tsc2*$^{+/-}$ mice (2). Next, we found that treatment with 1 μM Memantine over 4 hours produced a significant beneficial effect ($P < 0.05$ at 30 min, **Fig. 2B**). In addition, our initial set of experiments using NitroMemantine also demonstrated benefit (**Fig. 2C**).

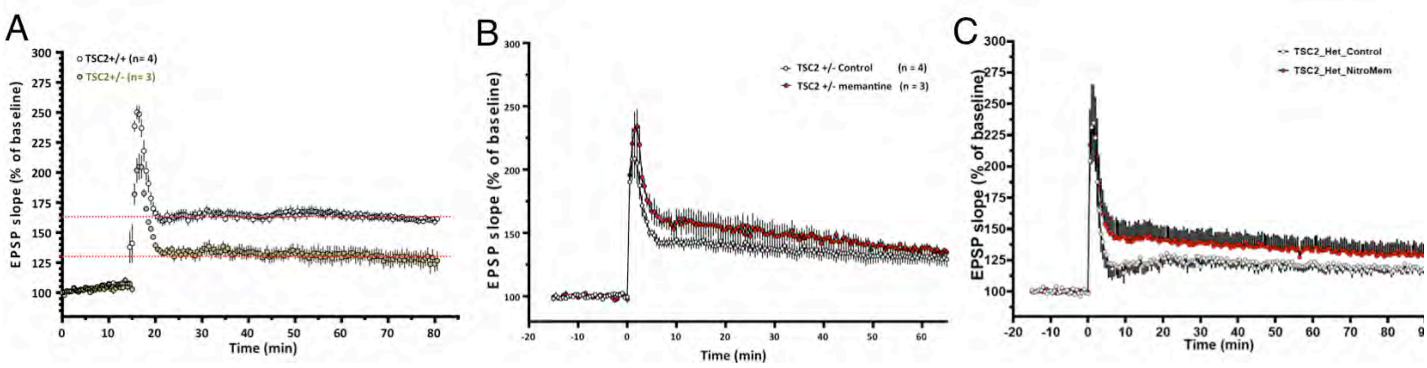

Fig. 2. Abnormal CA1-LTP in *Tsc2*$^{+/-}$ mice

(**A**) LTP recorded from hippocampal slices by MEA. Data are plotted every 30 sec and represent mean ± s.e.m. *Tsc2* $^{+/+}$, $n = 4$ slices total from 4 mice; *Tsc2* $^{+/-}$, $n = 3$ slices total from 3 mice.
(**B**) Effect of Memantine treatment (1 μM) on LTP in *Tsc2* $^{+/-}$ mice. *Tsc2* $^{+/-}$ with vehicle control, $n = 4$ slices total from 4 mice; *Tsc2* $^{+/-}$ with Memantine, $n = 3$ slices total from 3 mice.
(**C**) Effect of NitroMemantine treatment (1 μM) on LTP in *Tsc2* $^{+/-}$ mice 'Control' refers to treatment with vehicle rather than NitroMemantine (n =2 slices to date, but analysis is ongoing with additional slices).

To determine the pathogenic signaling cascades downstream of eNMDARs in *Tsc2*$^{+/-}$ mice, we investigated the p38 MAPK/MAPKAPK2 pathway using Western blotting, as we had proposed. However, we could not find any difference in the p38 MAPK/ MAPKAPK2 pathway between control and *Tsc2*$^{+/-}$ brains, suggesting an alternative approach was necessary to dissect out the abnormal eNMDAR signaling in *Tsc2*$^{+/-}$ mice.

Accomplishments with Discussion

The goal of this project was to determine if administration of the FDA-approved drug Memantine and its improved derivative, NitroMemantine, mitigates neurological manifestations in mouse models of TSC. Most importantly, we discovered that NitroMemantine offered additional benefit over Memantine in a neurobehavioral readout, the fear conditioning test, on *Tsc2*$^{+/-}$ mice. This result is in accord with our recent finding that NitroMemantine provides synaptic protection and rescues behavioral abnormalities to a greater extent than Memantine in Alzheimer's mouse models (3). Our new findings strongly support our original working hypothesis. In the future, additional multidisciplinary investigations, consisting of

electrophysiological, histological, biochemical, and behavioral approaches, will be necessary to evaluate and validate the efficacy of Memantine and NitroMemantine on the neurological manifestations of TSC.

4. KEY RESEARCH ACCOMPLISHMENTS

- Memantine treatment ameliorates and NitroMemantine normalizes deficits in the fear conditioning neurobehavioral test in *Tsc2*$^{+/-}$ mice.
- Memantine or NitroMemantine treatment can restore synaptic plasticity in *Tsc2*$^{+/-}$ mice, as monitored by electrophysiological recording of LTP.

5. CONCLUSION

The results of this proposal may have a major impact on TSC via new treatment for neurological manifestations, which are thought to be mediated in part by excessive glutamatergic activity in the brain. To this end, we tested the FDA-approved drug Memantine, which our group played a major role in developing for other neurological indications, as well as our improved derivatives, termed NitroMemantines. Since the quality of life for individuals with TSC is greatly affected by intellectual and neurological disabilities, it is important that we understand the pathogenesis of early cognitive deficits in order to develop rational and effective therapies for early postnatal or even embryonic treatment of TSC. Hence, we are hopeful that children with TSC will benefit from the research performed here via development of new drug therapies to increase their cognitive capacity and mitigate neurobehavioral abnormalities. Our results treating *Tsc2*$^{+/-}$ mice with Memantine and its improved analog NitroMemantine demonstrate promising beneficial effects. We are currently finishing our analysis of the data for publication. Based on these findings, we plan to apply for new grants from other funding agencies to confirm and extend our findings with Memantine and NitroMemantine.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

(1) Lay Press
http://cdmrp.army.mil/tscrp/research_highlights/13LiptonSulzerWongYoshii_highlight.shtml

7. INVENTIONS, PATENTS, AND LICENSES:

Nothing to report.

8. REPORTABLE OUTCOMES;

Treatment of TSC2 heterozygote mice with 1 μM Memantine or NitroMemantine for 4 hours improved long-term potentiation (LTP) in the CA1 region of the hippocampus. Previously, it had been shown that LTP, an electrophysiological correlate of learning and memory, was decreased in TSC2 heterozygous mice. We now report that treatment with Memantine (Namenda®), an FDA—approved drug that had been developed in our laboratory and licensed to Forest Laboratories in NYC, resulted in an increase in LTP in these mice. Additionally, TSC2 heterozygous mice show abnormalities in fear conditioning, a neurobehavioral test of hippocampal function. Treatment with Memantine mitigated and NitroMemantine restored the responses to fear conditioning in these mice.

9. OTHER ACHIEVEMENTS:

Nothing to report.

10. REFERENCES:

1. Auerbach BD, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature. 2011;480(7375):63-8. Epub 2011/11/25. doi: 10.1038/nature10658. PubMed PMID: 22113615; PubMed Central PMCID: PMC3228874.
2. Gkogkas C, Sonenberg N, Costa-Mattioli M. Translational control mechanisms in long-lasting synaptic plasticity and memory. J Biol Chem. 2010;285(42):31913-7. Epub 2010/08/10. doi: 10.1074/jbc.R110.154476. PubMed PMID: 20693284; PubMed Central PMCID: PMC2952191.

3. Talantova M, Sanz-Blasco S, Zhang X, Xia P, Akhtar MW, Okamoto S, Dziewczapolski G, Nakamura T, Cao G, Pratt AE, Kang Y-J, Tu S, Molokanova E, McKercher SR, Hires A, Sason H, Stouffer DG, Buczynski MW, Solomon J, Michael S, Powers ET, Kelly JW, Roberts AJ, Tong G, Fang-Newmeyer T, Parker J, Holland EA, Zhang D, Nakanishi N, Chen H-SV, Wolosker H, Parsons LH, Ambasudhan R, Masliah E, Heinemann SF, Piña-Crespo JC, Lipton SA. Aß induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. Proc Natl Acad Sci U S A. 2013;110(27):E2518-27. Epub 2013/06/19. doi: 10.1073/pnas.1306832110. PubMed PMID: 23776240; PubMed Central PMCID: PMC3704025.

11. APPENDICES:

Nothing to report.

APPENDIX

Personnel Receiving Pay from the Research Effort of this Award

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